

# An Id-related helix–loop–helix protein encoded by a growth factor-inducible gene

(inhibitor of DNA binding/transcription factors/cell proliferation/mouse chromosome 4)

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**ABSTRACT** An mRNA encoding a helix–loop–helix protein that we have named HLH462 is induced in mouse 3T3 cells as part of the immediate early transcriptional response to growth factors and other signaling agents. The RNA is present in a number of mouse tissues and in the developing mouse fetus. The HLH462 gene has been mapped by interspecific backcross analysis to the distal region of mouse chromosome 4. In its helix–loop–helix region HLH462 is closely related to the Id protein and the *Drosophila emc* protein. Like Id, HLH462 lacks a basic region required for DNA binding, and it inhibits the DNA-binding activities of other helix–loop–helix proteins. On the basis of its structural and functional similarity to Id, we suggest that HLH462 may inhibit the activities of helix–loop–helix transcription factors during the cellular growth response and during development.

When growth factors or other extracellular ligands bind to cell surface receptors, they induce sequential changes in gene expression in target cells. In mouse 3T3 cells the first genes activated by serum growth factors include those that code for a diverse group of transcription regulators: Jun and Fos family members, zinc-finger proteins, and serum response factor (SRF) (1, 2). Such immediate early transcription factors are thought to regulate genetic programs that mediate cellular responses to extracellular signals.

In this report we describe the induction in mouse 3T3 cells and the presence in the developing mouse fetus and in adult tissues of mRNA that codes for a protein (called HLH462) that belongs to a recently recognized class of transcription factors, the helix–loop–helix proteins (3). The distinguishing feature of this class is an amino acid sequence motif predicted to form a helix–loop–helix structure through which the proteins dimerize (3). Most members of the helix–loop–helix protein family so far described also have a basic DNA-binding domain adjacent to the dimerizing region, but others do not (4–6). Among the latter is the protein Id, implicated in the suppression of mammalian muscle cell differentiation (5) and the *Drosophila emc* protein, a negative regulator of sensory organ development on the fly's body surface (4, 6). These proteins are thought to act by forming inactive dimers with positively acting helix–loop–helix transcription factors (5). HLH462 shows extensive amino acid sequence similarity to Id and the *Drosophila emc* protein in the helix–loop–helix domain, and like Id it lacks a basic DNA-binding region but can interact with other helix–loop–helix proteins. Based on this structural and functional similarity, we hypothesize that HLH462 may negatively regulate the activity of other helix–loop–helix transcription factors during the cellular response to growth factors and during development.<sup>§</sup>

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## MATERIALS AND METHODS

**Cell Culture.** BALB/c 3T3 cells were maintained and stimulated with serum or other inducing agents as described (7).

**DNA Sequencing.** HLH462 cDNA and genomic DNA fragments were cloned into pBluescript II KS (Stratagene) and sets of 5' and 3' deletions were prepared. The resulting subclones were sequenced as double-stranded plasmids by the dideoxynucleotide chain-termination method (8) using 2'-deoxyadenosine 5'-[ $\alpha$ -<sup>35</sup>S]triphosphate and Sequenase (United States Biochemical).

**Primer Extension and S1 Nuclease Assay.** These procedures were carried out as described (9). The primer used was a <sup>32</sup>P-labeled oligonucleotide complementary to nucleotides 132–155 of the cDNA (Fig. 1). Partial sequence of the genomic fragment used to prepare the probe for S1 analysis is shown in Fig. 1.

**In Vitro Transcription and Translation.** *In vitro* transcription and translation (10) used either T7 or T3 RNA polymerase for transcription. The HLH462 cDNA template was a cDNA clone containing nucleotides 44–969 (see Fig. 1). cDNA clones for MyoD1 and E12 were generously supplied by H. Weintraub (Hutchinson Cancer Research Center, Seattle) and D. Baltimore (Rockefeller University, New York), respectively.

**RNA Blot Hybridization.** This procedure was performed as described (11) with 15  $\mu$ g of total cellular RNA per lane. DNA fragments used as probes were <sup>32</sup>P-labeled by nick-translation (Boehringer Mannheim).

**DNA-Binding Assays.** Double-stranded oligonucleotides corresponding to the MyoD binding site in the muscle creatine kinase (MCK) enhancer (5) or the Zif268 binding site (MUT.3, ref. 12) were labeled as described (12). Binding to the MCK enhancer site was carried out as described (5) except that sonicated salmon sperm DNA was used at 50  $\mu$ g/ml as nonspecific competitor, and reactions were carried out in a total volume of 25  $\mu$ l with 12.5 fmol of <sup>32</sup>P-labeled DNA probe. Binding to the Zif268 site was carried out as described (12) except that EDTA was omitted from the binding buffer. Competition assays were carried out in a total volume of 25  $\mu$ l with 5 fmol of labeled probe (12). Competitors used were double-stranded oligonucleotides corresponding to Zif268 sites (12) or the Zif-like site within the HLH462 promoter (Fig. 1). For competition analysis using the SRE-like site within the HLH462 promoter, HeLa cell nuclear extract was used as a source of SRF protein (13). Double-

Abbreviations: CRE, cAMP response element; MCK, muscle creatine kinase; PDGF, platelet-derived growth factor; SRE, serum response element; SRF, serum response factor.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M60523 and M60524).

CRE-like

GATCTTTAGTTGTTCAAGGGATTTATGACCTCAGA

-400

SRE-like

-365 GCTGTGGGTTTCGAACCAGTATTGGGGGGAGGGGACCTCAGAGAACTTGGGCGCTCCAGAAAAGGCATATTCTTAAAG

-286 TTAATGGTTTTAAGTGATTTTTTTTTTTTTTCAAATCTGTGCTGGCTCAAGAGCCCGCCTCTCCTCGGTATCAGCGCT

-207 TCCTCATTCCTCGCATCCGAGGCTCCGCTGTCTCGGCGTCAGACCAGCCTAAGGAAGCCTGTTAGCAATTTAAACCCA

Spl-like ZIF-like

-128 CTGTGAACGCCAGGACCGGGGAGCGGTGCCAGGGCAGCGGGGTGACCCCTGGCGGTCTGTTTGAATAAGGGG

▼

-49 GTGTGCTCTAGAGAGGACTCTATAAGAGTCGGCCGCTGCAGGCGTGCAGCCTGTTTGTGCTTTAGGTGTCTCTTTT

31 CCTCCCTCTCTATCTCTACTCTCCAAC ATG AAG GCG CTG AGC CCG GTG CGC GGC TGC TAC GAG GCG 13

44 CTCTACTCTCCAAC ATG AAG GCG CTG AGC CCG GTG CGC GGC TGC TAC GAG GCG  
met lys ala leu ser pro val arg gly cys tyr glu ala 13

97 GTG TGC TGC CTG TCG GAA CGT AGC CTG GCC ATT GCG CGA GGC CGC GGT AAG AGC CCG TCG  
val cys cys leu ser glu arg ser leu ala ile ala arg gly arg gly lys ser pro ser 33

157 ACC GAG GAG CCT CTT AGC CTC TTG GAC GAC ATG AAC CAC TGC TAC TCG CGC CTG CGG GAA  
thr glu glu pro leu ser leu leu asp asp met asn his cys tyr ser arg leu arg glu 53

217 CTG GTG CCG GGA GTC CCG CGA GGC ACT CAG CTT AGC CAG GTG GAA ATC CTG CAG CGT GTC  
leu val pro gly val pro arg gly thr gln leu ser gln val glu ile leu gln arg val 73

277 ATA GAC TAC ATC CTC GAC CTT CAG GTG GTC CTG GCA GAG CCG GCG CCT GGA CCC CCG GAC  
ile asp tyr ile leu asp leu gln val val leu ala glu pro ala pro gly pro pro asp 93

337 GGT CCG CAT CTC CCG ATC CAG ACA GCT GAG CTC ACT CCG GAA CTT GTG ATC TCC AAG GAC  
gly pro his leu pro ile gln thr ala glu leu thr pro glu leu val ile ser lys asp 113

397 AAG AGG AGC TTT TGC CAC TGA CCCGGTCGTCCTGGCACCTCCCGAACGCAGGTGCTGGCGCCCGTTCCGCTT  
lys arg ser phe cys his OPA 119

469 GGGACCCTGGGACTCTGGGACTCTGGGACCCCTCTCTCCAGCCCGAAGCCTGAGGGCATGGATGAGCTTCGATCTTAACC

548 CAGCCCTCTTCACTTACCTGAACCTCAACGCCTCGAGGCTGGACCTGGAGCCCGAGAGAAGGACTGAACTTGGGTGGCC

627 TGAAGAGCTAGCACACGCTGGTCAGCAGCTGGGCAACGTCACCTCTGTCCCCACCTGACTCAAGTCTAAAAGACTGGCT

706 TTTCCGAGAATGGGGTGTGAGAGGGTGTGGGGGATGCGAGTGCGCTGCCCTGCGCACTCTGCCAAGGCAGCATAAGAG

785 CTGTTCTTCTGGTTTCTTGGAGAAAAGCTCTGCTGCCCTGATTATGAACCTCTATAATAGATATATAGCTTTTGTACC

864 TTTTTTACAGGAAGGTGACTTTCTGTAATCATGTGATGTATATTAACTTTTATAAAAGTTAACATTTTGCATAATAA

943 ACCATTTTGAACAAAAA

FIG. 1. Nucleotide sequence of HLH462 cDNA and the upstream genomic region. (Upper) Sequence of the 5' genomic region of the HLH462 gene together with sequence overlapping the cDNA. Negative numbers refer to the number of nucleotides upstream of the transcription start site (arrowhead), as determined by primer extension and S1 nuclease analysis described in the text. Sequences that resemble known regulatory elements are boxed (CRE, cAMP response element; SRE, serum response element; see text for further explanation). The TATA-like element (positions -25 to -29) is underlined. (Lower) Nucleotide sequence and predicted amino acid sequence for the HLH462 cDNA. Numbers at left refer to the first nucleotide in each line. Numbers at right refer to the last amino acid codon in each line. The portion of the cDNA that encodes the potential helix-loop-helix domain is underlined. The opal termination codon (TGA) is indicated (OPA), and the polyadenylation signal is underlined.

stranded unlabeled oligonucleotides corresponding to the SRE-like site in the HLH462 promoter or a previously described double-stranded oligonucleotide site from the *c-fos* promoter (13) were preincubated with the extract before addition of the probe. Gel analysis of protein-DNA complexes was carried out as described (12, 13).

**Interspecific Backcross Mapping.** Interspecific backcross progeny were generated by mating (C57BL/6J  $\times$  *Mus spretus*)F<sub>1</sub> females and C57BL/6J males (14). A total of 205 N<sub>2</sub> progeny were obtained; a random subset of these N<sub>2</sub> mice were used to map the *Hlh462* locus (see text for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described (15). All blots were prepared with Zetabind nylon membrane (AMF Cuno). The *Hlh462* probe [the 940-base-pair (bp) mouse cDNA] was labeled with [ $\alpha$ -<sup>32</sup>P]dCTP by using a nick-translation kit (Boehringer Mannheim). Washing was generally done to a final stringency of 75 mM NaCl/7.5 mM sodium citrate, pH

7.4/0.1% SDS at 65°C. An  $\approx$ 8.2-kilobase (kb) fragment was detected in *Bam*HI-digested C57BL/6J DNA; an  $\approx$ 9.5-kb fragment was detected in *Bam*HI-digested *M. spretus* DNA. Probes and restriction fragment length polymorphisms for *Lmyc*, *Fgr*, *Dsi-1*, and *Anf* have been described (16). Recombination distances were calculated (17) using the computer program SPRETUS MADNESS, developed by D. Dave (Data Management Services, Frederick, MD) and A. M. Buchberg (National Cancer Institute-Frederick Cancer Research and Development Center). Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

## RESULTS AND DISCUSSION

**Nucleotide Sequence of HLH462 cDNA and 5' Flanking Genomic Sequence.** Lau and Nathans (7) previously reported the isolation of a cDNA (clone 3CH 462) derived from an immediate early mRNA of about 1 kb induced in BALB/c

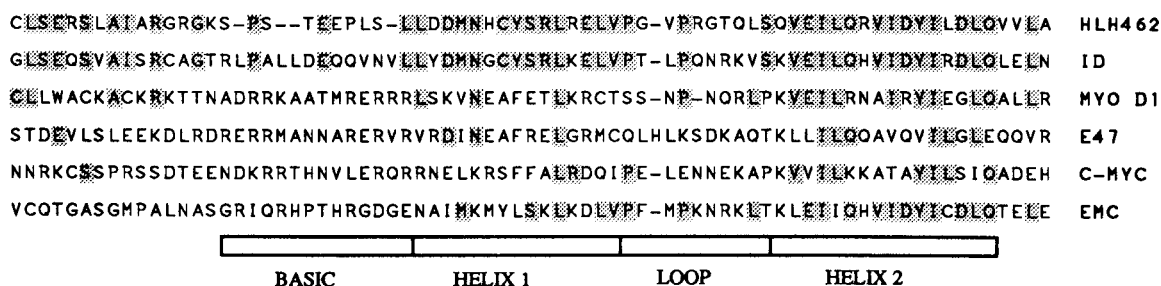


FIG. 2. Comparison of the predicted amino acid sequence of HLH462 with the sequences of selected helix-loop-helix proteins. Protein sequences are shown in the single-letter code. Amino acid identities relative to the HLH462 sequence are shaded. Sources of the other sequences: Id (5), MyoD1 (24), E47 (3), c-Myc (25), and Emc (4, 6). Except for Emc (*Drosophila*), all sequences are derived from murine cDNA clones.

3T3 cells by serum, platelet-derived growth factor (PDGF), or fibroblast growth factor. Using this partial cDNA as a probe, we isolated independent cDNA clones of  $\approx 1$  kb (hereafter called HLH462 cDNA) and sequenced each strand of one of these isolates and partially sequenced another. The sequence of 940 nucleotides has a single long open reading frame beginning at the first ATG and encodes 119 amino acids (Fig. 1). There is a poly(A)-addition signal 18 nucleotides from the poly(A) tail and four TTTA or ATTT sequences near the end of the cDNA that may be related to the short half-life of HLH462 mRNA (7, 18). The nucleotide sequence surrounding the first ATG triplet fits the consensus sequence for an efficient translation start signal (19).

The 5' end of the HLH462 mRNA missing in the cDNA clone was defined by reverse transcription of RNA from 3T3 cells stimulated with serum for 3 hr in the presence of cycloheximide, using an antisense oligonucleotide primer corresponding to nucleotides 155–132 of the cDNA (data not shown). These results were confirmed by S1 nuclease mapping using a  $^{32}$ P-labeled single-stranded DNA probe synthesized on an HLH462 cloned genomic fragment starting with the above-described primer (data not shown). We conclude from these experiments that HLH462 mRNA is 955 nucleotides in length exclusive of the poly(A) tail, and its predominant 5' end maps on the genomic sequence as shown in Fig. 1.

Also shown in Fig. 1 is the mouse genomic sequence extending to 400 nucleotides upstream of the transcription start site. There is a TATA sequence at –29, a possible Zif268 binding site at (12) at –88, two overlapping Sp1 sites (20) at –90 and –94 that also overlap the Zif268 site, a possible SRE (21, 22) at –351, and a possible CRE (23) at –375. To determine whether the SRE-like element and the Zif268-like site bind their respective proteins, synthetic oligonucleotides corresponding to these sequences were tested for their ability to inhibit the binding of SRF to the c-fos SRE (21, 22) or to inhibit the binding of Zif268 to its cognate site (12). In the case of the SRE-like sequence, an oligonucleotide containing this sequence failed to compete with the c-fos SRE for binding to SRF; therefore it is not likely to be a functional SRE. However, a double-stranded oligonucleotide (GATCCGC-GGGCGGGGGTGGATC) containing the putative Zif268 site upstream of the HLH462 gene did compete with a known site for binding by the Zif268 protein (data not shown). We conclude that, like a number of other immediate early genes, the HLH462 gene (*Hlh462*) has an upstream binding site for Zif268 and may be regulated by it.

**The HLH462 Protein.** The long open reading frame of HLH462 cDNA encodes a protein of 119 amino acids recognizable as a helix-loop-helix protein by comparison with other members of this family of transcription factors (Fig. 2). Between residues 40 and 81 HLH462 shows 69% identity with the helix-loop-helix sequence of Id (5) and clear relatedness to the helix-loop-helix sequences of MyoD (24), the *Drosophila emc* protein (4, 6), E47 (3), and c-Myc (25). Like Id and the *Drosophila emc* protein (4, 5), HLH462 lacks a

basic region, which in other helix-loop-helix proteins is required for specific binding to DNA (26, 27). Outside the protein regions shown in Fig. 2 there is only limited similarity between the amino acid sequence of HLH462 and those of the other proteins.

To determine whether the HLH462 protein interacts with other helix-loop-helix proteins as predicted from its amino acid sequence, we determined the effect of HLH462 synthesized *in vitro* on the DNA-binding activity of a mixture of MyoD and E12 (Fig. 3). HLH462 inhibited the binding of MyoD/E12 to an oligonucleotide containing the MCK enhancer sequence, but it had no effect on the binding of an unrelated protein (Zif268) to its binding site (12). We infer that HLH462, like Id (4), can dimerize with other helix-loop-helix proteins and inhibit their DNA-binding activity.

**Induction of HLH462 mRNA in 3T3 Cells.** The HLH462 gene is rapidly and transiently activated in BALB/c 3T3 cells by serum or PDGF, resulting in an increased mRNA level (ref. 7 and Fig. 4). As is the case with other immediate early mRNAs, HLH462 mRNA is superinduced by growth factor in the presence of inhibitors of protein synthesis, and its mRNA has a short half-life (7). Other agents also produce a rise in HLH462 mRNA in 3T3 cells (Fig. 4), including

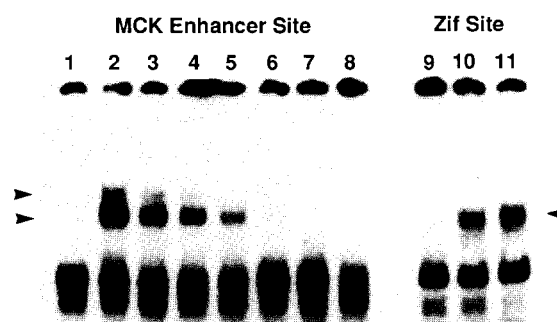


FIG. 3. Inhibition of binding of MyoD/E12 heterodimers to the MCK enhancer by HLH462. Electrophoretic mobility shift assays were performed using unlabeled proteins translated *in vitro*. The probe was  $^{32}$ P-labeled MCK enhancer oligonucleotide (lanes 1–8) or  $^{32}$ P-labeled Zif268 oligonucleotide (lanes 9–11). All reaction mixtures contained the same total amount of reticulocyte lysate. Lane 1, control reticulocyte lysate alone (not programmed with RNA); lane 2, MyoD1 plus E12 (approximately equal amounts); lanes 3–5, MyoD1 plus E12 preincubated with 2  $\mu$ l (lane 3), 4  $\mu$ l (lane 4), or 8  $\mu$ l (lane 5) of HLH462 translation product before addition of the labeled probe; lane 6, MyoD1 alone; lane 7, E12 alone; lane 8, HLH462 alone (8  $\mu$ l). Lanes 9–11 represent controls in which the effect of HLH462 on Zif268 binding to DNA was tested. The probe used was  $^{32}$ P-labeled Zif268 oligonucleotide (12). Lane 9, control reticulocyte lysate alone; lane 10, Zif268 translation product alone; lane 11, Zif268 preincubated with 8  $\mu$ l of HLH462 translation product before addition of probe. Arrowheads indicate specific protein-oligonucleotide complexes. Only the relevant part of the autoradiogram is shown.



Comparison of the interspecific backcross map with the composite intraspecific backcross map (16) indicates that *Hlh462* is located in a region of chromosome 4 in which several mutations have been localized. It will be interesting to determine whether any of these mutant animals show changes in the structure or expression of the HLH462 gene. We note also that the segment of mouse chromosome 4 to which *Hlh462* maps is homologous with the p36 region of human chromosome 1 (Fig. 6). Since translocation breakpoints associated with human neoplasia have been assigned to 1p36 (28), it will be important to determine if the human HLH462 gene maps to this locus and whether its expression is perturbed in neoplastic cells containing a 1p36 breakpoint.

## CONCLUSION

HLH462 resembles Id and the *Drosophila emc* protein in its helix-loop-helix domain and in its lack of a DNA-binding basic region. Like Id, it interacts with other HLH proteins and inhibits their DNA-binding activities. Therefore HLH462 would be expected to inhibit the transcriptional effects of helix-loop-helix transcription factors with which it can form heterodimers. In the context of the genetic program induced in fibroblasts by growth factors, HLH462 may dimerize with other helix-loop-helix proteins and thereby limit their activities. In view of the recent observations that expression of MyoD cDNA in cultured cells suppresses DNA synthesis induced by serum (29) and the proliferation of both normal and transformed cells (30), a specific hypothesis to be tested is that HLH462 antagonizes the actions of an endogenous growth-suppressing helix-loop-helix transcription factor present in quiescent cells. Similarly the observation that HLH462 mRNA is abundant in the early stages of embryonic development of the mouse and sharply decreases thereafter raises the possibility that HLH462 suppresses certain developmental processes until the later stages of fetal maturation. Insight into the role of HLH462 in cell proliferation and development may come from identifying proteins in quiescent cells and in relevant tissues of the early fetus that interact with HLH462.

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1. Bravo, R. (1989) in *Growth Factors, Differentiation Factors, and Cytokines*, ed. Habenich A. (Springer, New York).

2. Lau, L. F. & Nathans, D. (1990) in *Molecular Aspects of Cellular Regulation*, eds. Cohen, P. & Foulker, J. G. (Elsevier, Amsterdam), Vol. 6.
3. Murre, C., McCaw, P. S. & Baltimore, D. (1989) *Cell* **56**, 777-783.
4. Garrell, J. & Modolell, J. (1990) *Cell* **61**, 39-48.
5. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. & Weintraub, H. (1990) *Cell* **61**, 49-59.
6. Ellis, H. M., Spann, D. R. & Posakony, J. W. (1990) *Cell* **61**, 27-38.
7. Lau, L. F. & Nathans, D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1182-1186.
8. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.
9. Ryder, K., Lau, L. F. & Nathans, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1487-1491.
10. Pelletier, J. & Sonnenberg, N. (1985) *Cell* **40**, 515-526.
11. Linzer, D. I. H. & Nathans, D. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4271-4275.
12. Christy, B. & Nathans, D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8237-8241.
13. Christy, B. & Nathans, D. (1989) *Mol. Cell. Biol.* **9**, 4889-4895.
14. Buchberg, A. M., Bedigan, H. G., Taylor, B. A., Brownell, E., Ihle, J. N., Nagata, S., Jenkins, N. A. & Copeland, N. G. (1988) *Oncogene Res.* **2**, 149-165.
15. Jenkins, N. A., Copeland, N. G., Taylor, B. A. & Lee, B. K. (1982) *J. Virol.* **43**, 26-36.
16. Ceci, J. D., Siracusa, L. D., Jenkins, N. A. & Copeland, N. G. (1989) *Genomics* **5**, 699-709.
17. Green, E. L. (1981) *Genetics and Probability in Animal Breeding Experiments* (Macmillan, New York), pp. 77-113.
18. Shaw, G. & Kamen, R. (1986) *Cell* **46**, 659-667.
19. Kozak, M. (1987) *J. Mol. Biol.* **196**, 947-950.
20. Kadonaga, J. T., Jones, K. A. & Tjian, R. (1986) *Trends Biochem. Sci.* **11**, 20-23.
21. Treisman, R. (1985) *Cell* **42**, 889-902.
22. Treisman, R. (1986) *Cell* **46**, 567-574.
23. Montminy, M. R., Sevarino, K. A., Wagner, J. A., Mandel, G. & Goodman, R. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6682-6686.
24. Davis, R. L., Weintraub, H. & Lassar, A. B. (1987) *Cell* **51**, 987-1000.
25. Bernard, O., Cory, S., Gerondakis, S., Webb, E. & Adams, J. M. (1983) *EMBO J.* **2**, 2375-2383.
26. Davis, R. L., Cheng, P. F., Lassar, A. B. & Weintraub, H. (1990) *Cell* **60**, 773-746.
27. Voronava, A. & Baltimore, D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4722-4726.
28. Bloomfield, C. D. & Frestedt, J. L. (1990) in *Genetic Maps*, eds. O'Brien, S. J. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 186-196.
29. Sorrentino, V., Pepperkok, R., Davis, R. L., Ansorge, W. & Phillipson, L. (1990) *Nature (London)* **345**, 813-815.
30. Crescenzi, M., Fleming, T. P., Lassar, A. B., Weintraub, H. & Aaronson, S. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8442-8446.